Appendix 14

CONFIDENTIAL

ETAP
Ethologie Appliquée
Applied Ethology

ETAP CODE N°: INGREDIA 7/0698/T/PS-EDC

INGREDIA CODE N°: ING 913/TOLE/R-IP

CONFIDENTIEL

TOLERANCE TO THE ANXIOLYTIC EFFECT OF THE PRODUCT "ING 913" INTRAPERITONEALLY ADMINISTERED ON CONDITIONED DEFENSIVE BURYING IN THE MALE WISTAR RAT

FINAL REPORT

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Final Report Date: October 26, 1998

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PRECLINICAL STUDY (RATS)
(in vivo)

Final Report Date: October 26, 1998 ETAP Code N°: INGREDIA 7/0698/T/PS-EDC	Study Period: July, 1998 INGREDIA CODE N° ING 913/TOLE/R-IP

Title of the study: Tolerance to the anxiolytic effect of the product "ING 913", intraperitoneally administered, on Conditioned Defensive Burying in the male Wistar rat.

Experimental device: Habituation, shocking and testing were done in a 44 x 28 x 18 cm clear Plexiglas chamber, the floor of which was evenly covered with 5 cm of bedding material made of wood sawdust. On the center of one wall, 2 cm above the level of the bedding material, was a small hole through which a shock-probe could be inserted. The shock-probe was a craftsman's 7 x 2 x 0.5 cm Plexiglas probe overlayed with a copper wire-integrated circuit connected to a two-pole shocker.
The shock-probe was connected with an electric shock generator which delivered 0 to 8 mA.

Procedure: The Conditioned Defensive Burying test was performed for 5 minutes during the first five hours of the dark cycle, period where the rats were very active.
The shock-probe was inserted into the chamber before the test session. The rat was placed in the test chamber on the side opposite the shock-probe and the first time the rat touched it with its forepaws, the experimenter delivered a single 2-mA shock. Immediately after shock administration, the behavior of each rat was recorded on VHS-videotapes for 5 minutes.

Animals: Thirty-six male Wistar AF EOPS (Centre d'élevage Iffa-Credo, France), weighing 220 to 240 g.

Conditioning of animals: Rats were identified and housed in polycarbonate cages 48 x 27 x 20 cm in an air-conditioned room maintained at a relatively constant temperature (22 ± 2°C) and with a 12 hour light-dark cycle. Tap water and standard diet were available *ad libitum*. After an acclimatization period of 7 days after the day of arrival, the rats were weighed and randomly put into 3 groups. The rats of various groups were all handled in the same way and under the same conditions.

Test Substance: Product "ING 913"

Administration	Route	Frequency	Dose	Vehicle	Batch	Supplier
Four day pre-treatment period and one administration before the test	I.P.	Twice daily for 4 days; one administration 30 minutes before the test on day 5	1.2mg/kg for 4 days; 0.8 mg/kg before the test	NaCl 0.9% solution	Not communicated	INGREDIA France

Reference substance: Diazepam (Produits Roche, France, Batch number F042A) 1.5 mg/kg, i.p., twice daily for 4 days; 1 mg/kg, i.p., 30 minutes before the test on day 5.

Recorded variables:

- duration of probe-burying;
- number of head stretchings towards the probe;
- number of approaches towards the probe;
- number of retreats away from the probe.

The "number of approaches" and the "number of retreats" allow to calculate the "percentage of approaches towards the probe followed by retreats": $\frac{\text{Number of retreats}}{\text{Number of approaches}} \times 100$.

Method of evaluation:
Within each of the variables "duration of probe-burying", "number of head stretchings towards the probe" and "percentage of approaches towards the probe followed by retreats", all the values were classified in increasing order and then transformed in their respective ranks. For each rat, the sum of the ranks of the three variables represents its anxiety global score.

Statistics:
All the quantitative results were analysed using an analysis of variance followed by an unpaired t-test (2-tailed) to compare the product "ING 913" and diazepam groups with saline. Data were reported as mean ± s.e.m. (standard error of the mean). Differences were considered to be significant at $p < 0.05$.
All the statistical analyses were carried out using the Statview® 4.1 package.

RESULTS

At a dose of 0.8 mg/kg, i.p., the product "ING 913" significantly decreased the anxiety global score.

Diazepam (1 mg/kg, i.p.) did not significantly decrease the anxiety global score.

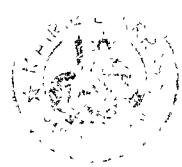
Effects of products on anxiety global score

Products	Saline	Diazepam	ING 913
Rats per group	12	12	12
Dose (mg/kg, i.p.)	-	1	0.8
Anova $F_{(2,33)} = 11.186$; $p < 0.001$			
Mean ± s.e.m.	62.46 ± 7.03	72.54 ± 6.91	31.50 ± 5.05
Unpaired t-test (vs. saline) Significance		t = 1.02 N.S.	t = 4.80 p < 0.005

CONCLUSION

In our experimental conditions, no tolerance to the product "ING 913" (0.8 mg/kg, i.p.) was apparent when the product was twice daily intraperitoneally administered at a dose of 1.2 mg/kg for four days.

Tolerance to diazepam (1 mg/kg, i.p.) was exhibited after four day pre-treatment at a twice daily dose of 1.5 mg/kg, i.p. in the Conditioned Defensive Burying model in the male Wistar rat.



CONTENTS

QUALITY ASSURANCE STATEMENT

STUDY: ETAP CODE N°: INGREDIA 7/0698/T/PS-EDC
INGREDIA CODE N°: ING 913/TOLE/R-IP

TITLE: Tolerance to the anxiolytic effect of the product "ING 913", intraperitoneally administered, on Conditioned Defensive Burying in the male Wistar rat.

I, the undersigned, hereby declare that the results presented in this report, in some cases recorded automatically and in others transcribed from the original data sheets, were verified by me item by item in comparison with the original data sheets.
To the best of my knowledge, there were no circumstances that may have affected the quality or integrity of the data.

October 26, 1998

Pr. D. DESOR, Scientific Adviser and Quality Assurance
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U.R.A. C.N.R.S. 1293 - 54500 Vandœuvre-lès-Nancy
Government authorization to perform experiments on live animals n° 04140/1991

AUTHORS

I, the undersigned, hereby declare that the work described in this report was performed under my supervision as Scientific Director and that the report provides a true and accurate record of the results obtained.
I declare that the present study was performed in accordance with ETAP's Standard Operating Procedures and in accordance with the principles of Good Laboratory Practice, including appropriate archiving of the original data sheets.
I declare further that the rats used in the present study were treated according to the rules provided by the ASAB Ethical Commettee (Guidelines for the use of animals in research; Animal Behavior, 45: 209-212, 1993).

October 26, 1998

M. MESSAOUDI, Scientific Director
Biology of Behavior - Ethopharmacology
Government authorization to perform experiments on live animals n° 04535/1991

We, the undersigned, responsible for the execution of the experiments described in this report hereby, declare that the experiments were performed as described and that the data presented correspond exactly to the results obtained during the experiments.

October 26, 1998

V. GRASMÜCK, Study Director
Biology of Behavior

F. DESOR, Research Technician
Biology of Behavior

The present study was carried out in ETAP's Research Center - 40, rue Lionnois F-54000 Nancy - Government authorization n° A54805.

1 - SUMMARY

TOLERANCE TO THE ANXIOLYTIC EFFECT OF THE PRODUCT "ING 913" INTRAPERITONEALLY ADMINISTERED ON CONDITIONED DEFENSIVE BURYING IN THE MALE WISTAR RAT

Conditioned Defensive Burying test was used to assess the phenomenon of tolerance to the product "ING 913" in the male Wistar rat after chronic administration. the product "ING 913" was dissolved in 0.9% NaCl solution and twice daily i.p. administered at a dose of 1.2 mg/kg for 4 days. On day 5, the product was administered at a dose of 0.8 mg/kg, i.p. 30 minutes before burying test.
Diazepam was used as a reference substance. It was suspended in 0.9% NaCl solution and i.p. twice daily administered at a dose of 1.5 mg/kg for 4 days. On day 5, the product was administered at a dose of 1 mg/kg, i.p. 30 minutes before test.

At a dose of 0.8 mg/kg, the product "ING 913" significantly decreased the anxiety global score.
Diazepam (1 mg/kg) did not significantly decrease the anxiety global score.

Tolerance to diazepam (1 mg/kg, i.p.) was exhibited after four day pre-treatment at a twice daily dose of 1.5 mg/kg, i.p. in the Conditioned Defensive Burying model in the male Wistar rat.
No tolerance to the product "ING 913" (0.8 mg/kg, i.p.) was apparent when the product was intraperitoneally twice daily administered at a dose of 1.2 mg/kg for four days before the test.

2 - INTRODUCTION

ETAP-Ethologie Appliquée was asked by INGREDIA to assess the phenomenon of tolerance to the product "ING 913" in the male Wistar rat after chronic administration. The model employed was the Conditioned Defensive Burying test, a model of natural defensive reaction in the rat.

The product "ING 913" was dissolved in 0.9% NaCl solution and twice daily i.p. administered at a dose of 1.2 mg/kg for 4 days. On day 5, the product was administered at a dose of 0.8 mg/kg, i.p. 30 minutes before burying test.

Diazepam was used as a reference substance. It was suspended in 0.9% NaCl solution and i.p. twice daily administered at a dose of 1.5 mg/kg for 4 days. On day 5, it was administered at a dose of 1 mg/kg, i.p. 30 minutes before the test.

3 - MATERIALS AND METHODS

3.1 - Conditioned Defensive Burying test

The Conditioned Defensive Burying (CDB) used in the present study is based on Pinel and Treit's procedure (1978, 1981). Traditional animal models use complex learning paradigms based on unatural responses and are not appropriate for studying anxiety. Today we observe an increasing interest for species-typical behavior to evaluate the emotional state of animals. Burying is part of the rat's natural repertoire; it requires no training and is elicited by aversive stimulation. Several investigators, having shown that anxiolytics decreased or suppressed burying, suggested that CDB might have advantages as a preclinical screening method for anti-anxiety agents.

3.2 - Animals

Thirty-six male Wistar AF EOPS (Centre d'élevage Iffa-Credo, 69 - St-Germain sur l'Arbresle, France), weighing 220 to 240 g were used. On receipt the rats were identified and then housed in polycarbonate cages 48 x 27 x 20 cm (U.A.R., 91 - Epinay-Sur-Orge, France) in an air-conditioned room maintained at a relatively constant temperature (22 ± 2°C) and with a 12 hour light-dark cycle. Tap water and standard diet (food pellets M25, Ets Piétrement, 77 - Provins, France) were available *ad libitum*.

After an acclimatization period of one week after the day of arrival, the rats were weighed and randomly put into 3 groups (n = 12). The rats of various groups were all handled in the same way and under the same conditions.

3.3 - Materials

3.3.1 - Apparatus

Habituation, shocking and testing were done in a 44 x 28 x 18 cm clear Plexiglas chamber, the floor of which was evenly covered with 5 cm of bedding material made of wood sawdust. On the center of one wall, 2 cm above the level of the bedding material, was a small hole through which a shock-probe could be inserted.

3.3.2 - Shock-probe

The shock-probe was a craftsman's 7 x 2 x 0.5 cm Plexiglas probe overlayed with a copper wire-integrated circuit connected to a two-pole shocker.

3.3.3 - Electric material

The shock-probe was connected with an electric shock generator which delivered 0 to 8 mA (OPEN-Systems, 54 - Maxéville, France). The release was manually handled by the operator.

3.3.4 - Recorded behavioral sequences

In the slightly lit test room, a CCD-TV camera allowed the rats to be observed and filmed on VHS-videotapes from a neighbouring room.

3.4 - Products (Tab. 1)

Table 1

Products

Products	Saline	Diazepam	ING 913
Origin	Cooper France	Produits Roche France	INGREDIA France
Batch number	23331	F042A	Not communicated
Storage	At 4°C. protected from the light	At 4°C. protected from the light	At 4°C. protected from the light

3.5 - Procedure

The test was performed and the recorded behaviors were scored by experimenters unaware of the administered products.

3.5.1 - Habituation

Each home cage group was placed in the test chamber without the shock-probe for 20 minutes during the two days before testing commenced. The bedding material was changed and smoothed to a uniform depth of 5 cm before each group habituation period.

3.5.2 - Burying test

The Conditioned Defensive Burying test was performed for 5 minutes during the first 5 hours of the dark cycle, period where the rats were very active.

The shock-probe was inserted into the chamber before the test session. The rat was placed in the test chamber on the side opposite the shock-probe and the first time the rat touched it with its forepaws, the experimenter delivered a single 2-mA shock. Immediately after shock administration, the behavior of each rat was recorded for 5 minutes.

The bedding material was changed before each group test period and was smoothed to a uniform depth of 5 cm before each rat test.

A subject that was not shocked after 5 minutes or appeared not to receive a shock upon touching the probe, was discarded from the study.

Because of the biological rhythms of rats, the study was performed on two consecutive days with 6 rats in each treatment group being tested in each day session test.

3.6 - Administration of products (Tab. 2)

The treatments were randomly pre-attributed by a computer system in each series of three animals.

The product "ING 913" and diazepam were dissolved or suspended in 0.9% NaCl solution on a magnetic stirrer for 30 minutes. The compounds were prepared just before their administration and i.p. injected 30 minutes prior to the test.

Table 2

Administration of products

Products	Rats per group	Chronic administration (mg/kg, i.p.) twice daily for 4 days	Testing dose (mg/kg, i.p.)	Volume (ml/kg)	Administration before the test (minutes)
Saline	12	-	-	2	30
Diazepam	12	1.5	1	2	30
ING 913	12	1.2	0.8	2	30

3.7 - Variables

- duration of probe-burying;
- number of head stretchings towards the probe;
- number of approaches towards the probe;
- number of retreats away from the probe.

The "number of approaches" and the "number of retreats" allow to calculate the "percentage of approaches towards the probe followed by retreats": $\frac{\text{Number of retreats}}{\text{Number of approaches}} \times 100$.

3.8 - Statistics

Within each of the variables "duration of probe-burying", "number of head stretchings towards the probe" and "percentage of approaches towards the probe followed by retreats", all the values were classified in increasing order and then transformed in their respective ranks. For each rat, the sum of the ranks of the three variables represents its anxiety global score.

All the quantitative results were analysed using an analysis of variance followed by an unpaired t-test (2-tailed) to compare the treated groups with saline. Data were reported as mean ± s.e.m. (standard error of the mean). Differences were considered to be significant at $p < 0.05$.

All the statistical analyses were carried out using the Statview® 4.1 package.

4 - RESULTS

4.1 - Anxiety global score (Tab. 3 & Fig. 1)

At a dose of 0.8 mg/kg, the product "ING 913" significantly decreased the anxiety global score.

Diazepam (1 mg/kg) did not significantly decrease the anxiety global score.

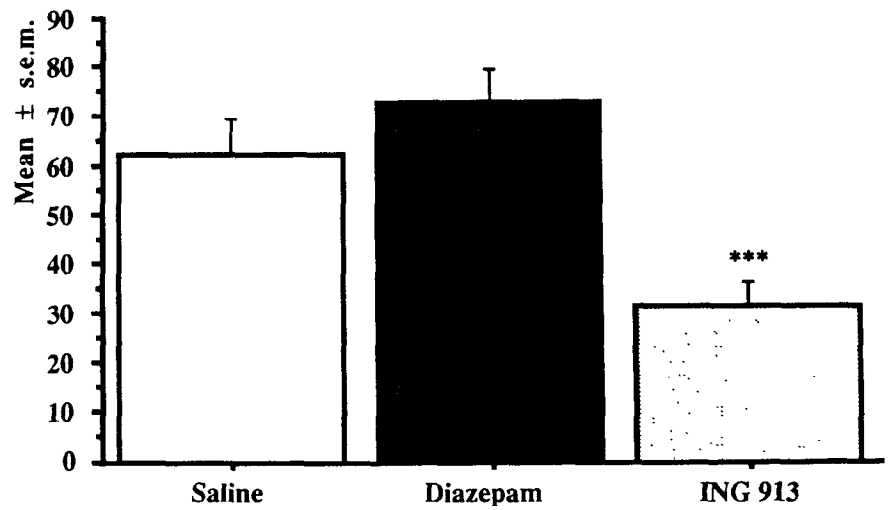
Table 3

Effects of products on anxiety global score

Products	Saline	Diazepam	ING 913
Rats per group	12	12	12
Dose (mg/kg, i.p.)	-	1	0.8
Anova $F_{(2,33)} = 11.19$; $p < 0.001$			
Mean ± s.e.m.	62.46 ± 7.03	72.54 ± 6.91	31.50 ± 5.05
Unpaired t-test (vs. saline) Significance		t = 1.02 N.S.	t = 4.80 p < 0.005

Figure 1

Effects of products on anxiety global score



Unpaired t-test: *** $p < 0.005$ (vs. saline)

5 - CONCLUSION

Conditioned Defensive Burying test was used to assess the phenomenon of tolerance to the product "ING 913" in the male Wistar rat after chronic administration. The product "ING 913" was dissolved in 0.9% NaCl solution and twice daily i.p. administered at a dose of 1.2 mg/kg for 4 days. On day 5, the product was administered at a dose of 0.8 mg/kg, i.p. 30 minutes before burying test.
Diazepam was used as a reference substance. It was suspended in 0.9% NaCl solution and i.p. twice daily administered at a dose of 1.5 mg/kg for 4 days. On day 5, the product was administered at a dose of 1 mg/kg, i.p. 30 minutes before test.

At a dose of 0.8 mg/kg, the product "ING 913" significantly decreased the anxiety global score.
Diazepam (1 mg/kg) did not significantly decrease the anxiety global score.

In our experimental conditions, tolerance to diazepam (1 mg/kg, i.p.) was exhibited after four day pre-treatment at a twice daily dose of 1.5 mg/kg, i.p. in the Conditioned Defensive Burying model in the male Wistar rat.
No tolerance to the product "ING 913" (0.8 mg/kg, i.p.) was apparent when the product was intraperitoneally twice daily administered at a dose of 1.2 mg/kg for four days before the test.

6 - ARCHIVES STATEMENT

Raw data, protocol and final report were kept in the archives room of ETAP for five years and VHS-videotapes for one year.

7 - REFERENCES

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8 - APPENDICES

8.1 - Statistic results

8.1.1 - Duration of probe-burying (Tab. 4)

At a dose of 0.8 mg/kg, the product "ING 913" significantly decreased the duration of probe-burying.

Diazepam (1 mg/kg) did not significantly decrease the duration of probe-burying.

Table 4

Effects of products on the duration of probe-burying (seconds)

Products	Saline	Diazepam	ING 913
Rats per group	12	12	12
Dose (mg/kg, i.p.)	-	1	0.8
Anova $F_{(2,33)} = 4.23$; $p = 0.02$			
Mean ± s.e.m.	48.92 ± 16.69	55.17 ± 14.46	6.33 ± 3.71
Unpaired t-test (vs. saline) Significance		t = 0.28 N.S.	t = 2.49 p < 0.05

8.1.2 - Number of head stretchings towards the probe (Tab. 5)

At a dose of 0.8 mg/kg, the product "ING 913" significantly decreased the number of head stretchings towards the probe.

Diazepam (1 mg/kg) did not significantly decrease the number of head stretchings towards the probe.

Table 5

Effects of products on the number of head stretchings towards the probe

Products	Saline	Diazepam	ING 913
Rats per group	12	12	12
Dose (mg/kg, i.p.)	-	1	0.8
Anova $F_{(2,33)} = 6.81$; $p = 0.003$			
Mean ± s.e.m.	4.67 ± 1.17	6.33 ± 0.93	1.50 ± 0.65
Unpaired t-test (vs. saline) Significance		t = 1.11 N.S.	t = 2.37 p < 0.05

8.1.3 - Percentage of approaches towards the probe followed by retreats (Tab. 6)

At a dose of 0.8 mg/kg, the product "ING 913" significantly decreased the percentage of approaches followed by retreats away from the probe.

Diazepam (1 mg/kg) did not significantly decrease the percentage of approaches followed by retreats away from the probe.

Table 6

Effects of products on the percentage of approaches towards the probe followed by retreats

Products	Saline	Diazepam	ING 913
Rats per group	12	12	12
Dose (mg/kg, i.p.)	-	1	0.8
Anova $F_{(2,33)} = 9.94$; $p < 0.001$ Mean ± s.e.m.	55.30 ± 7.54	62.95 ± 7.59	23.94 ± 3.83
Unpaired t-test (vs. saline) Significance		t = 0.72 N.S.	t = 3.71 p < 0.005

8.2 - Individual results

Products	Duration of burying (s)	Number of head stretchings	Number of approaches	Number of retreats
Saline	0	4	18	7
Saline	67	7	18	7
Saline	1	2	16	6
Saline	0	0	25	7
Saline	38	3	16	6
Saline	9	8	11	6
Saline	160	5	10	10
Saline	48	1	2	2
Saline	91	10	13	10
Saline	151	2	7	4
Saline	22	13	26	48
Saline	0	1	24	6
Diazepam	1	7	24	13
Diazepam	26	9	21	10
Diazepam	25	5	24	18
Diazepam	108	7	16	11
Diazepam	59	4	5	3
Diazepam	0	9	12	7
Diazepam	78	9	19	15
Diazepam	125	6	9	9
Diazepam	0	0	16	1
Diazepam	13	3	16	5
Diazepam	110	12	19	16
Diazepam	117	5	11	10
ING 913	0	0	20	1
ING 913	0	0	16	2
ING 913	0	0	22	3
ING 913	2	7	32	12
ING 913	0	2	34	7
ING 913	41	0	27	7
ING 913	4	0	20	5
ING 913	1	0	40	8
ING 913	0	2	30	6
ING 913	0	0	21	3
ING 913	4	3	11	5
ING 913	24	4	19	9

8.3 - Study proposal

STUDY PROPOSAL N° INGREDIA 7/0698/T/PS-EDC

TOLERANCE TO THE ANXIOLYTIC EFFECT OF THE PRODUCT "ING 913" INTRAPERITONEALLY ADMINISTERED ON CONDITIONED DEFENSIVE BURYING IN THE MALE WISTAR RAT

INTRODUCTION

ETAP-Ethologie Appliquée is asked by INGREDIA to assess the phenomenon of tolerance to the product "ING 913" in the male Wistar rat after chronic administration. The model employed is the Conditioned Defensive Burying test, a model of natural defensive reaction in the rat.

The product "ING 913" is dissolved in 0.9% NaCl solution and twice daily i.p. administered at a dose of 1.2 mg/kg for 4 days. On day 5, the product is administered at a dose of 0.8 mg/kg, i.p. 30 minutes before test.

Diazepam is used as a reference substance. It is suspended in 0.9% NaCl solution and i.p. twice daily administered at a dose of 1.5 mg/kg for 4 days. On day 5, the product is administered at a dose of 1 mg/kg, i.p. 30 minutes before test.

MATERIALS AND METHODS

Conditioned Defensive Burying test

The Conditioned Defensive Burying (CDB) used in the present study is based on Pinel and Treit's procedure (1978, 1981). Traditional animal models use complex learning paradigms based on unatural responses and are not appropriate for studying anxiety. Today we observe an increasing interest for species-typical behavior to evaluate the emotional state of animals. Burying is part of the rat's natural repertoire; it requires no training and is elicited by aversive stimulation. Several investigators, having shown that anxiolytics decreased or suppressed burying, suggested that CDB might have advantages as a preclinical screening method for anti-anxiety agents.

Animals

Thirty-six male Wistar AF EOPS (Centre d'élevage Iffa-Credo, 69 - St-Germain sur l'Arbresle, France), weighing 220 to 240 g are used. On receipt the rats are identified and then housed in polycarbonate cages 48 x 27 x 20 cm (U.A.R., 91 - Epinay-Sur-Orge, France) in an air-conditioned room maintained at a relatively constant temperature (22 ± 2°C) and with a 12 hour light-dark cycle. Tap water and standard diet (food pellets M25, Ets Piétrement, 77 - Provins, France) are available *ad libitum*.

After an acclimatization period of 7 days after the day of arrival, the rats are weighed and randomly put into 3 groups (n = 12). The rats of various groups are all handled in the same way and under the same conditions.

Materials

Apparatus

Habituation, shocking and testing are done in a 44 x 28 x 18 cm clear Plexiglas chamber, the floor of which is evenly covered with 5 cm of bedding material made of wood sawdust. On the center of one wall, 2 cm above the level of the bedding material, is a small hole through which a shock-probe could be inserted.

Shock-probe

The shock-probe is a craftsman's 7 x 2 x 0.5 cm Plexiglas probe overlayed with a copper wire-integrated circuit connected to a two-pole shocker.

Electric material

The shock-probe was connected with an electric shock generator which can deliver 0 to 8 mA (OPEN-Systems, 54 - Maxéville, France). The release is manually handled by the operator.

Recorded behavioral sequences

In the slightly lit test room, a CCD-TV camera allows the rats to be observed and filmed on VHS-videotapes from a neighbouring room.

Procedure

The test is performed and the recorded behaviors are scored by experimenters unaware of the administered products.

Habituation

Each home cage group is placed in the test chamber without the shock-probe for 20 minutes during the two days before testing commenced. The bedding material is changed and smoothed to a uniform depth of 5 cm before each group habituation period.

Burying test

The Conditioned Defensive Burying test is performed for 5 minutes during the first 5 hours of the dark cycle, period where the rats are very active.

The shock-probe is inserted into the chamber before the test session. The rat is placed in the test chamber on the side opposite the shock-probe and the first time the rat touchs it with its forepaws, the experimenter delivers a single 2-mA shock. Immediately after shock administration, the behavior of each rat is recorded for 5 minutes.

The bedding material is changed before each group test period andis smoothed to a uniform depth of 5 cm before each rat test.

A subject that is not shocked after 5 minutes is discarded from the study.

Because of the biological rhythms of rats, the study is performed on two consecutive days with 6 rats in each treatment group being tested in each day session test.

Administration of products

The treatments are randomly pre-attributed by a computer system in each series of 3 rats.

The product "ING 913" and diazepam are dissolved or suspended in 0.9% NaCl solution on a magnetic stirrer for 30 minutes. The compounds are prepared just before their i.p. administration 30 minutes prior to the test.

Table 1

Administration of products

Products	Rats per group	Chronic administration (mg/kg, i.p.) twice daily for 4 days	Testing dose (mg/kg, i.p.)	Volume (ml/kg)	Administration before the test (minutes)
Saline	12	-	-	2	30
Diazepam	12	1.5	1	2	30
ING 913	12	1.2	0.8	2	30

Variables

- duration of probe-burying;
- number of head stretchings towards the probe;
- number of approaches towards the probe;
- number of retreats away from the probe.

The "number of approaches" and the "number of retreats" allow to calculate the "percentage of approaches towards the probe followed by retreats": $\frac{\text{Number of retreats}}{\text{Number of approaches}} \times 100$.

Statistics

Within each of the variables "duration of probe-burying", "number of head stretchings towards the probe" and "percentage of approaches towards the probe followed by retreats", all the values are classified in increasing order and then transformed in their respective ranks. For each rat, the sum of the ranks of the three variables represents its anxiety global score.

All the quantitative results are analysed using an analysis of variance followed by an unpaired t-test (2-tailed) to compare the treated groups with saline. Data are reported as mean ± s.e.m. (standard error of the mean). Differences are considered to be significant at $p < 0.05$.

All the statistical analyses are carried out using the Statview® 4.1 package.

ARCHIVES STATEMENT

Raw data, protocol and final report are kept in the archives room of ETAP for five years and VHS-videotapes for one year after the delivery of the study report.

EXPERIMENTAL PERIOD

July, 1998.

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PROTOCOL APPROVAL

TOLERANCE TO THE ANXIOLYTIC EFFECT OF THE PRODUCT "ING 913" INTRAPERITONEALLY ADMINISTERED ON CONDITIONED DEFENSIVE BURYING IN THE MALE WISTAR RAT

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